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(54) Title: MELANOCORTIN RECEPTOR-3 LIGANDS TO TREAT SEXUAL DYSFUNCTION

(57) Abstract: Methods for treating sexual dysfunction, such as erectile dysfunction or sexual arousal disorder, with a compound having the generic formula X_1-X_2-(D)Phe-Arg-(D)Trp-X_3. A particularly useful compound is HP-228, which has the formula Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2. The invention also provides methods for selecting melanocortin receptor-3 ligands by determining whether a compound modulates the activity of MC-3 as an agonist or antagonist. These methods can be used to screen compound libraries for ligands to treat MC-3-associated conditions. Such conditions include sexual dysfunction, including erectile dysfunction and sexual arousal disorder.

MELANOCORTIN RECEPTOR-3 LIGANDS TO TREAT SEXUAL DYSFUNCTION

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to melanocortin receptors and more specifically to the treatment of sexual dysfunction using melanocortin receptor 3 ligands.

BACKGROUND INFORMATION

Sexual dysfunction can be due to several physiological, as well as psychological, factors. In males, erectile dysfunction can be associated with diseases such as diabetes mellitus, syphilis, alcoholism, drug dependency, hypopituitarism and hypothyroidism. Erectile dysfunction can also be caused by vascular and neurogenic disorders, or be a side effect of drugs such as hypertensives, sedatives, tranquilizers and amphetamines. In all, erectile dysfunction is estimated to affect up to 10 million men in the United States, with its incidence increasing with age up to 25% of men at age 65.

While various pharmaceutical treatments are commercially available or being developed, the underlying physiological bases for sexual dysfunction are not well understood. Attention has recently been drawn to melanocortin (MC) receptors, which are a group of cell surface proteins that mediate a variety of physiological effects. The MC receptors have been implicated in the regulation of adrenal gland function such as production

of the glucocorticoid cortisol and aldosterone, control of melanocyte growth and pigment production, control of feeding, thermoregulation, immunomodulation, inflammation and analgesia. Five distinct MC receptors have been cloned, although the specific role of each MC receptor is still unclear.

Certain compounds, termed "melanocortins" have been found to bind MC receptors, causing the activity of the receptors to increase or decrease. These melanocortins include melanocyte-stimulating hormones (MSH) such as α-MSH, β-MSH and γ-MSH, as well as adrenocorticotropic hormone (ACTH). Other compounds may bind as ligands to MC receptors, increasing or decreasing the activity of the receptors.

Thus, there is a need for compounds that can affect the activity of specific MC receptors that are involved with sexual dysfunction. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a method for treating sexual dysfunction, such as erectile dysfunction or sexual arousal disorder, with a compound having the generic formula X_1-X_2-(D)Phe-Arg-(D)Trp-X_3. A particularly useful compound is HP-228, which has the formula Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2.

The invention also provides methods for selecting melanocortin receptor-3 ligands by determining whether a compound modulates the activity of MC-3 as an agonist or antagonist. These methods can be used to screen compound libraries for ligands to treat MC-3-associated conditions. Such conditions include sexual dysfunction, including erectile dysfunction and sexual arousal disorder.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows a reaction scheme for the combinatorial synthesis of benzimidazole derivative compounds.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method for treating sexual dysfunction. The term "sexual dysfunction" herein means any condition that inhibits or impairs normal sexual function, including coitus. However, the term need not be limited to physiological conditions, but may include psychogenic conditions or perceived impairment without a formal diagnosis of pathology.

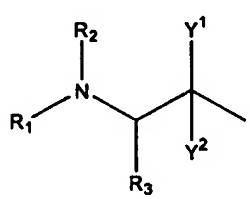
In males, sexual dysfunction includes erectile dysfunction. The term "erectile dysfunction" or "impotence" means herein the inability or impaired ability to attain or sustain an erection that would be of satisfactory rigidity for coitus. Sexual dysfunction in males can also include premature ejaculation and priapism, which is a condition of prolonged and sometimes painful erection unrelated to sexual activity, often associated with sickle-cell disease.

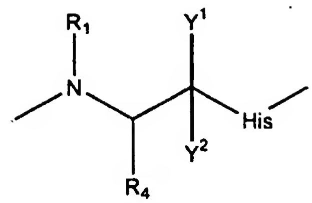
In females, sexual dysfunction includes sexual arousal disorder. The term "sexual arousal disorder" means herein a persistent or recurrent failure to attain or maintain the lubrication-swelling response of sexual excitement until completion of sexual activity. Sexual dysfunction in females can also include inhibited orgasm and dyspareunia, which is painful or difficult coitus. Sexual dysfunction can also be manifested as inhibited sexual desire or inhibited lordosis behavior in animals.

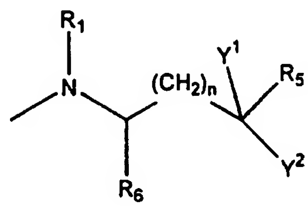
The method for treating sexual dysfunction comprises the step of administering to the subject an effective dose of the compound

$$X_1\text{-}X_2\text{-(D)Phe-Arg-(D)Trp-}X_3$$

wherein

X_1 is , $COCH_3$, H or absent;

X_2 is , His, $COCH_3$ or H; and

X_3 is  or R_5; wherein

R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, COO-t-butyl, $COOCH_2Ph$, CH_2CO-(polyethylene glycol) or A;

R_2 is H, $COCH_3$, C_2H_5 or CH_2Ph;

R_3 is a linear alkyl group having 1 to 6 carbon atoms or a branched or cyclic alkyl group having 3 to 6 carbon atoms;

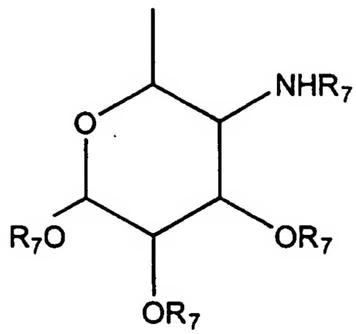
R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA;

R_5 is OH, OR_3, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A;

R_6 is H or R_3;

R_7 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, COO-t-butyl, $COOCH_2Ph$ or CH_2CO-(polyethylene glycol);

Ph is C_6H_5; m is 1, 2 or 3; n is 0, 1, 2 or 3; Y^1 and Y^2 are independently hydrogen atoms, or are taken together to form a carbonyl or thiocarbonyl; and A is



These peptide compounds are characterized in part by the core structures (D)Phe-Arg-(D)Trp or His-(D)Phe-Arg-(D)Trp and are described in U.S. Patent No. 5,420,109, issued May 30, 1995, and U.S. Patent No. 5,726,156, issued March 10, 1998.

Particular compounds can be selected by further defining one or more of the individual variables in the generic formula above. The variable X_1 can be norleucine or Ac-norleucine; or even norvaline, Ac-norvaline, leucine, Ac-leucine, isoleucine or Ac-isoleucine. The variable X_2 can be Gln-His or His. The variable X_3 can be Gly or Gly-NH_2. The variable R_7 can be H, C_2H_5 or CH_2Ph. The variables R_1 and R_2 can be $COCH_3$ or H independently. The variable R_5 can be NH_2. The variable R_5 can be covalently bound to X_1, forming a cyclic peptide.

A particularly useful compound is HP-228, which has the formula Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2. HP-228 can be synthesized as described in Example I.A. The effectiveness of HP-228 in treating sexual dysfunction such as erectile dysfunction is demonstrated in Example II. In addition, Example VII shows that the erectile effect of HP-228 is not significantly affected

by antagonists to oxytocin receptors or antagonists to dopamine receptors (D2, D3).

Other useful compounds can have the (D)Phe of the formula be iodinated in the *para* position. Thus, a useful compound is HP-467, which has the formula Ac-Nle-Gln-His-(*para*-iodo-(D)Phe)-Arg-(D)Trp-Gly-NH_2. HP-467 can be prepared as further described in Example I.B.

Other specific compounds useful in the method of the invention include

(D)Phe-Arg-(D)Trp
Ac-(D)Phe-Arg-(D)Trp
(D)Phe-Arg-(D)Trp-NH_2
Ac-(D)Phe-Arg-(D)Trp-NH_2

(cyclohexyl)Gly-Gln-His-(D)Phe-Arg-(D)Trp-Gly
Ac-(cyclohexyl)Gly-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2
cyclo(His-(D)Phe-Arg-(D)Trp)

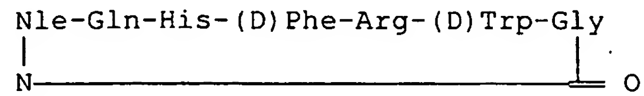
Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2
His-(D)Phe-Arg-(D)Trp-Gly
His-(D)Phe-Arg-(D)Trp-Gly-NH_2
Ac-His-(D)Phe-Arg-(D)Trp-NH_2
His-(D)Phe-Arg-(D)Trp-OH
His-(D)Phe-Arg-(D)Trp
His-(D)Phe-Arg-(D)Trp-NH_2
Ac-His-(D)Phe-Arg-(D)Trp-OH
Ac-His-(D)Phe-Arg-(D)Trp-Gly-NH_2

Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-OH
Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-OC_2H_5
Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH-NH_2
Ac-Nle-Asn-His-(D)Phe-Arg-(D)Trp-Gly-NH_2

Ac-Nle-Asn-His-(D)Phe-Arg-(D)Trp-Gly-OH

Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-$NHCH_2CH_2Ph$

Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-$NHCH_2Ph$



Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2

Ac-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2

Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-NH_2

Ac-His-(D)Phe-Arg-(D)Trp(CH_2)-(NAc)Gly-NH_2

His-(D)Phe-Arg-(D)Trp(CH_2)-(NAc)Gly

Amino acids are indicated by their commonly known three-letter code. Nle is the three-letter code for norleucine. The prefix (D)- designates an amino acid having the D-configuration, as opposed to the naturally occurring L-configuration. Where no specific configuration is indicated, a skilled artisan would understand the amino acid to be an L-amino acid. Finally, Ph indicates a phenyl group (C_6H_5).

A skilled artisan would know that the choice of amino acids or amino acid analogs incorporated into the compound will depend in part on the specific physical, chemical or biological characteristics required of the compound. Such characteristics can be determined by the route of administration and the desired location of action.

Selective modification of the reactive groups also can impart desirable characteristics to the compound. During synthesis, compounds can be manipulated while still attached to a resin to obtain N-terminal

modified compounds such as an acetylated peptide or can be removed from the resin using hydrogen fluoride or an equivalent cleaving reagent and then modified. Compounds synthesized containing the C-terminal carboxyl group (Wang resin) can be modified after cleavage from the resin or prior to solution phase synthesis.

Methods for modifying the N-terminus or C-terminus of a peptide are well known in the art and include methods for acetylating the N-terminus or amidating the C-terminus. Similarly, methods for modifying side chains of the amino acids or amino acid analogs are well known to those skilled in the art of peptide synthesis. The choice of modifications made to the reactive groups present on the peptide will be determined by the desired characteristics.

Cyclic peptides can also be compounds useful in the method of the invention. A cyclic peptide can be obtained by inducing the formation of a covalent bond between the amino group at the N-terminus of the peptide and the carboxyl group at the C-terminus. For example, the peptide cyclo(His-(D)Phe-Arg-(D)Trp) can be produced by forming a covalent bond between His and (D)Trp. A cyclic peptide can also be obtained by forming a covalent bond between a terminal reactive group and a reactive amino acid side chain or between two reactive amino acid side chains. One skilled in the art would know that the choice of a particular cyclic peptide is determined by the reactive groups present on the peptide as well as the desired characteristic of the peptide. For example, a cyclic peptide may provide a compound with increased stability *in vivo*.

The compound can also be administered to the subject by any number of routes known in the art. These routes include injection parenterally, such as intravenously (i.v.) for systemic administration, intramuscularly, subcutaneously, intraorbitally, intracapsularly, intraperitoneally (i.p.), intracisternally, intra-articularly. Other routes include orally, intravaginally, rectally, or oral or topical intubation, which can include direct application of an ointment or powder, or using a nasal spray or inhalant, which may include a propellant.

The compound can be administered through the skin by passive absorption such as a skin patch or facilitated absorption such as transdermal iontophoresis. Particular routes include transdermal delivery by passage through the skin into the blood stream and transmucosal delivery through mucosal tissue. Another route is transurethral or intraurethral, where the compound contacts and passes through the wall of the urethra and enters the blood stream.

The compound can also be incorporated into liposomes, microspheres or other polymer matrices (Gregoriadis, Liposome Technology, Vols. I to III, 2nd ed. (CRC Press, Boca Raton FL (1993)). Liposomes, which consist of phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

An "effective dose" of the compound herein means an amount of the composition that is sufficient to therapeutically alleviate a sexual dysfunction in a

subject or to prevent or delay onset or recurrence of the dysfunction.

The amount of a therapeutically effective dose depends on a variety of factors, including the particular characteristics of the compound, the type and severity of the sexual dysfunction and the patient's medical condition. Based on such factors, a skilled physician can readily determine a therapeutically effective dose of the compound, which can be about 0.0001 to 100 mg/kg body weight per administration. For example, the compound can be administered at 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 or 100 mg/kg body weight.

The total amount of compound can be administered as a single dose as a bolus or by infusion over a relatively short period of time, or can be administered in multiple doses administered over a more prolonged period of time. One skilled in the art would know that the amount of a compound depends on many factors including the age and general health of the subject as well as the route of administration and the number of treatments to be administered. In view of these factors, the skilled artisan would adjust the particular dose to obtain an effective dose for treating sexual dysfunction.

A compound can be administered to a subject as a pharmaceutical composition comprising the compound and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include aqueous solutions such as physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil or injectable organic esters.

A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that stabilize the compound or increase its absorption. Such physiologically acceptable compounds include carbohydrates such as glucose, sucrose or dextrans; antioxidants, such as ascorbic acid or glutathione; chelating agents; low molecular weight proteins or other stabilizers or excipients. One skilled in the art would know that the choice of a pharmaceutically acceptable carrier depends on the route of administration of the compound and on its particular physico-chemical characteristics.

The compounds used in the method of the invention can interact with melanocortin (MC) receptors to affect their activity. Five distinct MC receptors have been cloned and are known to mediate a variety of physiological effects. For example, MC-1 is involved in pain and inflammation. The MC receptors have also been implicated in the regulation of weight control, adrenal gland function such as production of the glucocorticoid cortisol and aldosterone, control of melanocyte growth and pigment production, thermoregulation, immunomodulation and analgesia.

The diversity of physiological responses to MC receptor signaling can be used to alter or regulate a physiological pathway that mediates or moderates a pathological condition or disease. Thus, the binding of an MC receptor ligand to an MC receptor can be used to modulate physiological responses.

The recent elucidation of the role of various MC receptors in particular physiological pathways supports the use of ligands that activate specific MC

receptors to modulate a physiological effect associated with a given condition or disease. The α-MSH analog MELANOTAN-II, which is an MC receptor ligand, has been shown to cause penile erections in human subjects in pilot phase I clinical studies (Dorr et al., Life Sciences 58:1777-1784 (1996); Wessells et al., J. Urology 160:389-393 (1998)). Due to the lack of ligands specific for particular MC receptors, however, the specific receptor associated with erectogenesis has been uncertain (Vergoni et al., Eur. J. Pharmacol. 362:95-101 (1998)).

The present invention discloses that the receptor MC-3 is specifically associated with sexual dysfunction. As discussed in Examples VI and VIII, this is demonstrated using compounds that act as MC-3-specific antagonists. Therefore, ligands for MC-3 that can alter the activity of an MC-3 receptor can be useful for treating sexual dysfunction and other conditions or conditions associated with MC-3 (*see* Getting et al., J. Immunol. 162:7446-7453 (1999)).

Other MC-3-associated conditions that can be treated with the MC-3 receptor ligands include disuse deconditioning; organ damage such as organ transplantation or ischemic injury; adverse reactions associated with cancer chemotherapy; diseases such as atherosclerosis that are mediated by free radicals and nitric oxide action; bacterial endotoxic sepsis and related shock; adult respiratory distress syndrome; and autoimmune or other patho-immunogenic diseases or reactions such as allergic reactions or anaphylaxis, rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, glomerulonephritis, systemic lupus erythematosus, transplant atherosclerosis and parasitic mediated immune dysfunctions such as Chagas's Disease.

Accordingly, the present invention provides a method for selecting an MC-3 ligand by initially contacting a compound with an MC-3 receptor. It is then determined whether the compound modulates the activity of the receptor. The compound is selected if the compound modulates the activity of the receptor.

A variety of assays can be used to measure activity modulation by MC-3 receptor ligands. Because MC receptors are G-protein-coupled receptors that activate adenylate cyclase and produce cAMP in response to binding of ligands, a cAMP assay can be useful for determining whether a compound can modulate an MC-3 receptor's activity. Such as assay is described in Example IV.

A particular MC-3 ligand can modulate the receptor's activity as an agonist. The term "agonist" means herein a ligand that increases or otherwise stimulates the activity of the MC-3 receptor. An increase in activity can be detected in a cAMP assay by elevation in cAMP compared to a negative control. The potency of the agonist can be represented in terms of EC_{50}, which is the concentration of ligand necessary to achieve 50% of the maximum response by that agonist in an assay. Thus, an MC-3 agonist can have an EC_{50} less than 1 μM. More potent agonists can have an EC_{50} less than 500, 200, 100 or 50 nM. HP-228, which is a powerful MC-3 agonist, can be used as a positive control for the assay. Thus, the MC-3 ligand can also be measured in comparison with HP-228, having at least 25%, 50%, 75% or even 100% of the MC-3 stimulatory effect of HP-228 in the assay. An example of an agonist is TRG 2411 #203, as described in Example II.A.

A particular MC-3 ligand can also act as an antagonist. The term "antagonist" herein means a ligand that binds to an MC-3 receptor, resulting in any decrease in the receptor's activity. The decrease can be determined in a cAMP assay by detecting a reduction in the stimulatory effect of HP-228. The potency of the antagonist can also be represented in terms of K_i (inhibitory constant) or EC_{50}, where EC_{50} is the concentration of ligand necessary to achieve 50% of the maximum decrease by that antagonist in an assay. Similarly, the potency of an antagonist can be expressed in terms of decrease in the potency of a given agonist as expressed by EC_{50}.

An MC-3 antagonist can be particularly useful for decreasing an MC-3-associated condition. For example, where penile erections are mediated by MC-3, decreasing the MC-3 activity using an MC-3 antagonist can be useful for treating priapism or otherwise reducing the ability of a subject to achieve or maintain an erection.

While a ligand that binds MC-3 and modulates MC-3 activity is useful, the same ligand may also be able to bind other MC receptors. If so, the ligand may also modulate the activity of those other receptors to some degree. While the presence of non-MC-3 activity may not necessarily be detectable or interfere with the intended effect of an MC-3 ligand, it can be desirable to select an MC-3 ligand so that such non-MC-3 activity is minimized. Thus, a particularly useful ligand can also show preferential or selective activity for MC-3 compared to other melanocortin receptors.

Thus, the selection method described above can further comprise the step of determining whether the

ligand is MC-3-preferring compared to a second melanocortin receptor. The compound can then be selected only if the compound is both an MC-3 ligand and MC-3-preferring. The term "second melanocortin receptor" as used herein can be any one or combination of MC-1, MC-2, MC-4, MC-5, or any other known melanocortin receptor other than MC-3.

A ligand can be "MC-3-preferring" in two ways, as illustrated in Example V. First, an agonist can be MC-3-preferring if the ligand has a lower EC_{50} for MC-3 than for the second MC. For example, the ligand's EC_{50} for MC-3 can be less than 5%, 10%, 20%, 50% or 100% of the ligand's EC_{50} for the second MC, as measured by a cAMP assay. Similarly, an MC-3-preferring antagonist can have a lower EC_{50} for MC-3 than for the second MC.

A ligand agonist or antagonist can also be MC-3-preferring by binding more tightly to MC-3 than to another melanocortin receptor. For example, the ability of a ligand compound to compete for binding of a known MC receptor ligand can be used to assess the affinity and specificity of the compound for one or more MC receptors. An example of such a competition assay is presented in Example III.

Any MC receptor ligand can be used so long as the competing ligand can be labeled with a detectable moiety. The detectable moiety can be a radiolabel, fluorescent label or chromophore, or any detectable functional moiety so long as the MC receptor ligand exhibits specific MC receptor binding. A particularly useful detectable MC receptor ligand for identifying and characterizing other MC receptor ligands is ^{125}I-HP 467,

which has the amino acid sequence Ac-Nle-Gln-His-(*para*-^{125}iodo-(D)Phe)-Arg-(D)Trp)-Gly-NH_2.

The binding affinity of a ligand for an MC receptor can be expressed as an IC_{50} value, which is the concentration giving 50% inhibition of binding of ^{125}I-HP 467. Thus, an MC-3-preferring ligand can have a lower IC_{50} for MC-3 than for the second MC. For example, the ligand's IC_{50} for MC-3 can be less than 5%, 10%, 20%, 50% or 100% of the ligand's IC_{50} for the second MC.

While an MC-3 ligand can have similar EC_{50} and IC_{50} profiles across several MC receptors, the profiles can also be different due to different receptor sources used in the assays. For example, EC_{50} or IC_{50} profiles may be determined using human or nonhuman rat/mouse MC receptors. Thus, both activity and binding preferences, as manifested by EC_{50} and IC_{50} profiles, can be separately useful for determining the MC-3 preference of a ligand. In addition, MC preferences may also differ when the profiles are determined *in vivo* or *in vitro*.

The invention also provides a method for screening a library of compounds for MC-3 ligands by selecting the compounds from the library for MC-3 modulating activity, as described above. The library can have at least 50, 100, 200, 500 or even 1000 compounds. Peptide and small molecule libraries have been described extensively in the literature and can be generated by combinatorial chemistry or other methods or can be obtained commercially.

Compounds obtained by screening such libraries may not be immediately administerable to a subject and may require an additional pharmaceutically acceptable

carrier, as described above. In particular, the ligand may not be readily soluble or be able to reach the intended target area in effective concentrations. For example, the ligand may not readily cross the blood-brain barrier upon administration. One skilled in the art will recognize that numerous methods are known in the art to solubilize and administer initially insoluble compounds, such as dissolving the compound in 20% DMSO or 20% CDEX dextran (w/v). As also discussed above, these solubility and other considerations will be recognized by the skilled artisan when determining the effective dose of the compound when used to treat an MC-3-associated condition.

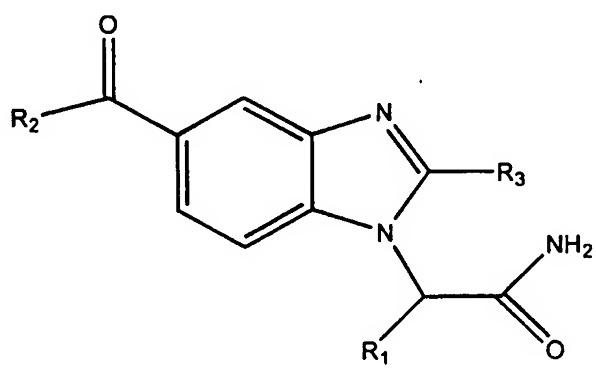
The invention further provides a method for treating an MC-3-associated condition in a subject. The term "MC-3-associated condition" includes any condition or condition mediated by MC-3 or can be affected by binding an MC-3 ligand. Such conditions include inflammation and sexual dysfunction.

The method for treating MC-3-associated conditions comprises administering to the subject an effective dose of a pharmaceutical composition, which comprises a pharmaceutically acceptable carrier and a compound obtained by screening a compound library, as described above. Induction of penile erections is demonstrated in Example II using compounds such as HP-228 as described in Example I.

An "effective dose" of the compound herein means an amount of the composition that is sufficient to therapeutically alleviate the MC-3-associated condition, such as sexual dysfunction, in a subject or to prevent or delay onset or recurrence of the condition.

The amount of a therapeutically effective dose depends on a variety of factors, including the particular characteristics of the compound, the type and severity of the MC-3-associated condition and the patient's medical condition. Based on such factors, a skilled physician can readily determine a therapeutically effective dose of the compound, which can be about 0.0001 to 100 mg/kg body weight per administration. For example, the compound can be administered at 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 or 100 mg/kg body weight.

Useful compounds obtained from screening libraries include benzimidazoles, which have the generic starting structure



The R_1, R_2 and R_3 positions can then be derivatized with compounds having functional groups using standard organic chemistry techniques. The variable R_1 can be derivatized with an amino acid. The variable R_2 can be derivatized with a primary or secondary amine. The variable R_3 can be derivatized with an aldehyde.

These compounds can be prepared as set forth in Figure 1 and as described below and in Example IX. Various benzimidazole derivative compounds can be

prepared. For instance, an N-protected amino acid can be coupled to an amine compound and then deprotected, resulting in a carboxamido-substituted amino compound having a substituent of the formula -NH-C(O)-variable group-NH_2. Alternatively, a diamine containing a variable group can be coupled to an amine compound in the presence of carbonyldiimidazole (CDI), resulting in an ureido-substituted amino compound having a substituent of the formula -NH-C(O)-NH-variable group-NH_2.

The amine compound can be attached to solid support, such as a functionalized resin (e.g., methylbenzhydrylamine (MBHA). Alternatively, a Merrifield resin can be coupled with a primary amine, resulting in the resin attached to a substituent of the formula -HN-variable group. Subsequently, the substituent can be coupled with an amino acid resulting in a group of the formula -HN-variable group-C(O)-variable group.

The carboxamido substituted amino compound can then be coupled to a phenyl compound with a nitro and a halo group at ortho positions, resulting in a phenyl compound substituted with a nitro group and an ortho-monosubstituted amino group. The phenyl compound being coupled can also have one to four additional substituents, such as carboxyl, halo, alkyl, etc. (see Figure 1).

Where the phenyl compound also has a carboxyl substituent, this substituent can be reacted with a (i) monosubstituted amine; (ii) disubstituted amine; (iii) cyclic imide; or (iv) alcohol; resulting, respectively, in a (i) monosubstituted carboxamido substituent; (ii) disubstituted carboxamido substituent;

(iii) cyclic imido carbonyl substituent; or (iv) ester substituent attached to the phenyl compound (see Figure 1). It should be understood that such a substituent can be at any one to four of the available positions on the phenyl ring.

The nitro group of the phenyl compound can be reduced. The resulting compound can be coupled with an aldehyde compound and cleaved (see Figure 1).

In addition, after cleaving, the amino group can be substituted. For example, the amino group can be alkylated with an alkyl halide or substituted alkyl halide.

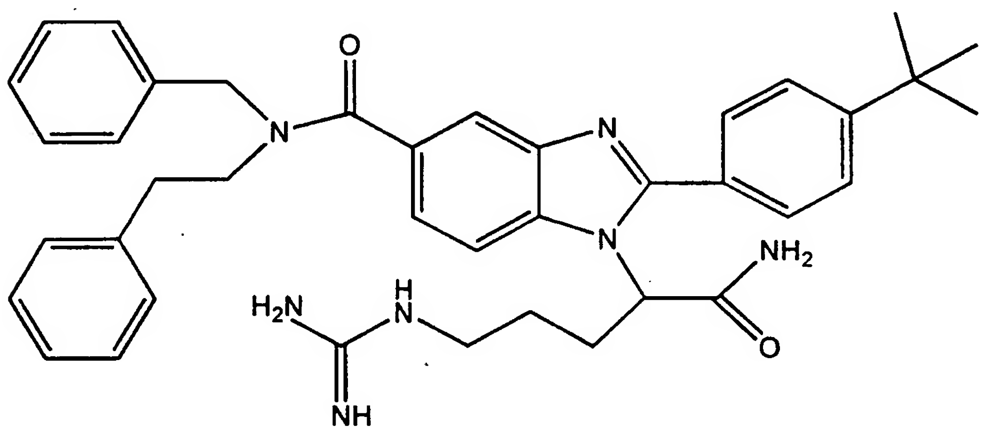
Resin-bound benzimidazole derivative compounds can be cleaved by treating them with HF gas. The compounds can be extracted from the spent resin with AcOH, for example (see Figure 1).

Particular compounds can be selected by further defining one or more of the individual variables in the generic formula above. Thus, R_1 can be derivatized with arginine; R_2 can be derivatized with phenethylbenzylamine or 1,2-diphenylethylamine; and R_3 can be derivatized with 4-*t*-butylbenzaldehyde, 4-*i*-propylbenzaldehyde or 4-butoxybenzaldehyde.

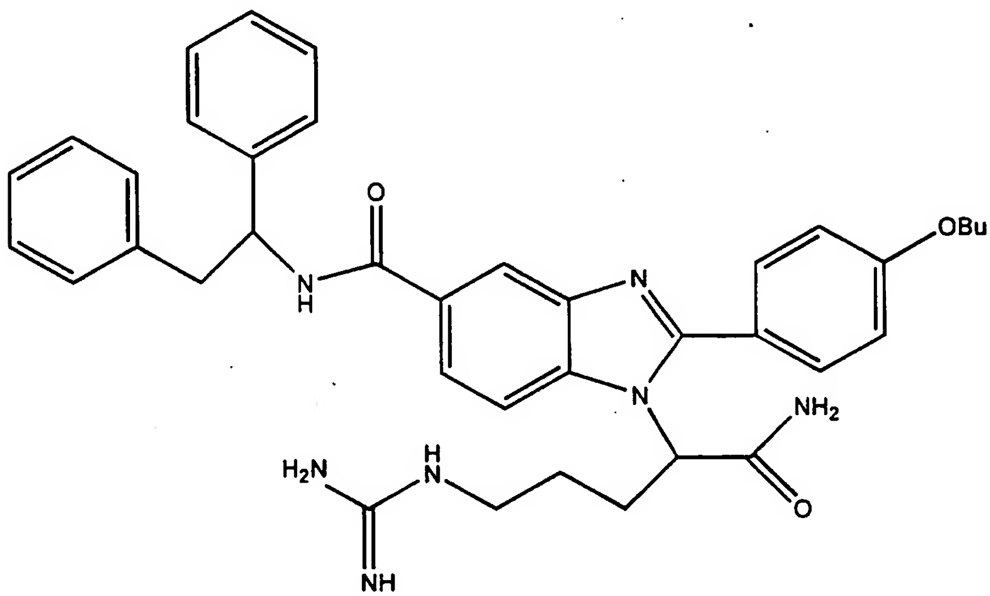
Specific benzimidazole compounds are designated Compounds A to E as follows. In each of the compounds, where the specific configuration of a chiral atom is not shown, all possible configurations are intended to be encompassed within the illustration. Illustration of a particular configuration is intended to show a preferred configuration; however, all configurations are still

intended to be illustrated, not limited to the particular configuration shown.

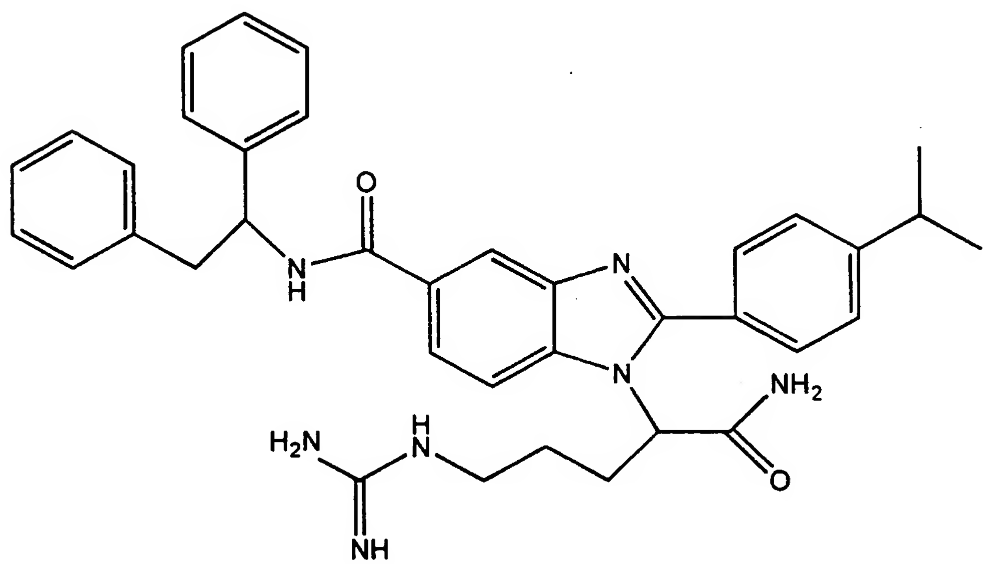
In Compound A, R_1 is derivatized with arginine, R_2 is derivatized with phenethylbenzylamine and R_3 is derivatized with 4-*t*-butylbenzaldehyde:



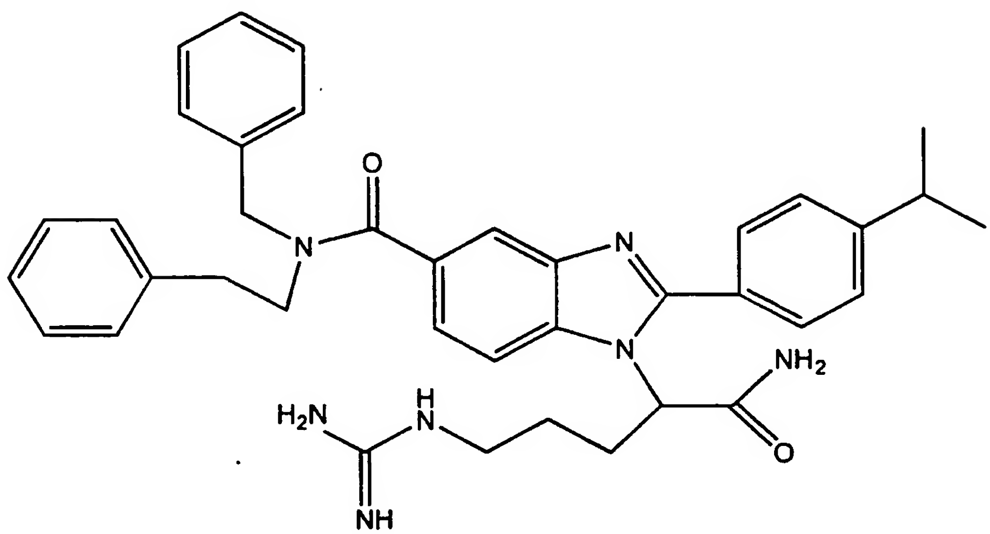
In Compound B, R_1 is derivatized with arginine, R_2 is derivatized with 1,2-diphenylethylamine and R_3 is derivatized with 4-butoxybenzaldehyde:



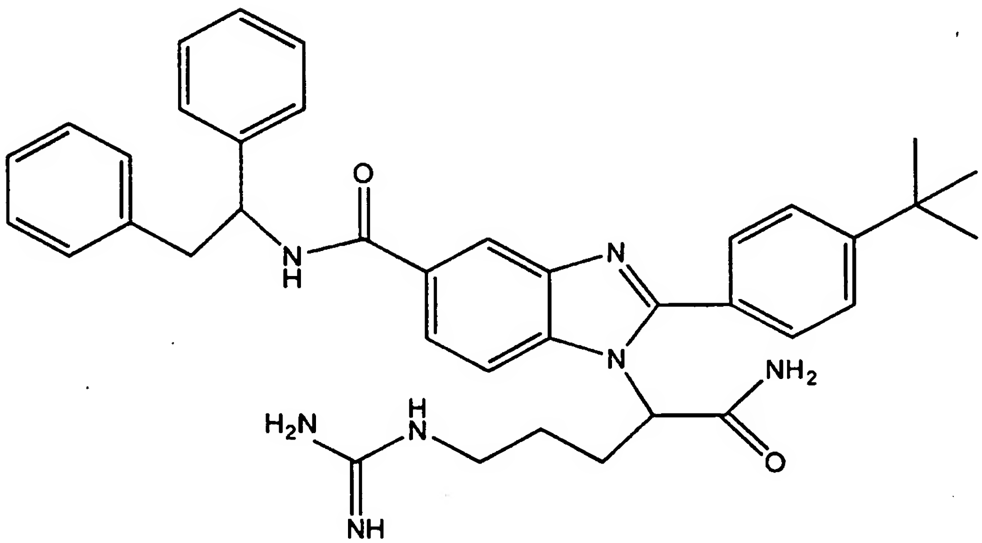
In Compound C, R_1 is derivatized with arginine, R_2 is derivatized with 1,2-diphenylethylamine and R_3 is derivatized with 4-*i*-propylbenzaldehyde:



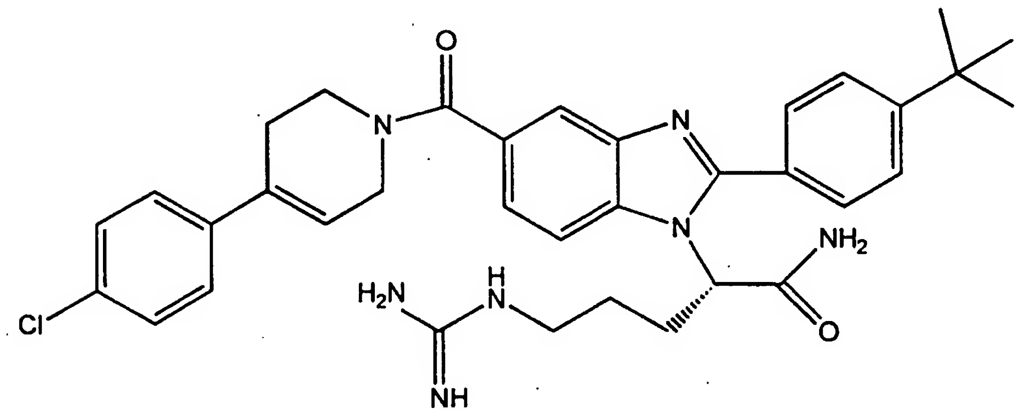
In Compound D, R_1 is derivatized with arginine, R_2 is derivatized with phenethylbenzylamine and R_3 is derivatized with 4-*i*-propylbenzaldehyde:



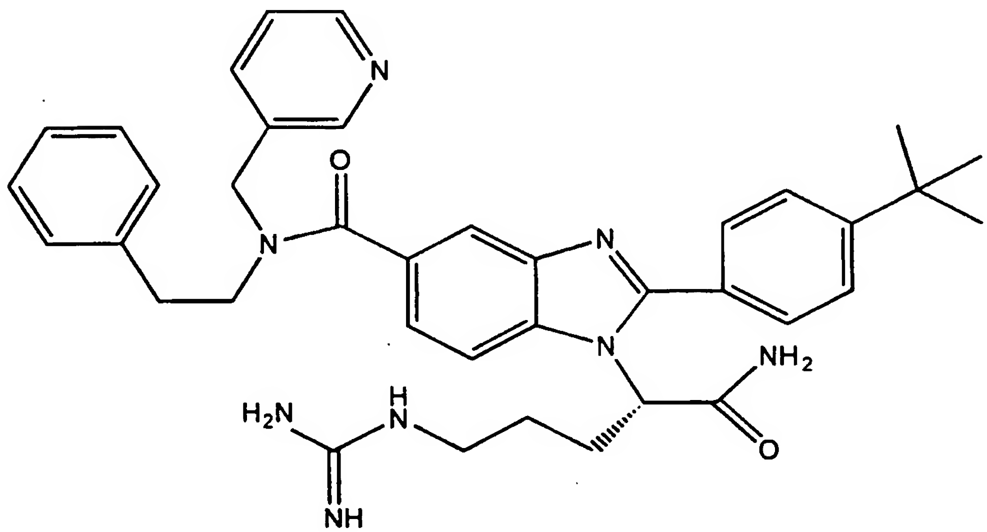
In Compound E, R_1 is derivatized with arginine, R_2 is derivatized with 1,2-diphenylethylamine and R_3 is derivatized with 4-*t*-butylbenzaldehyde:



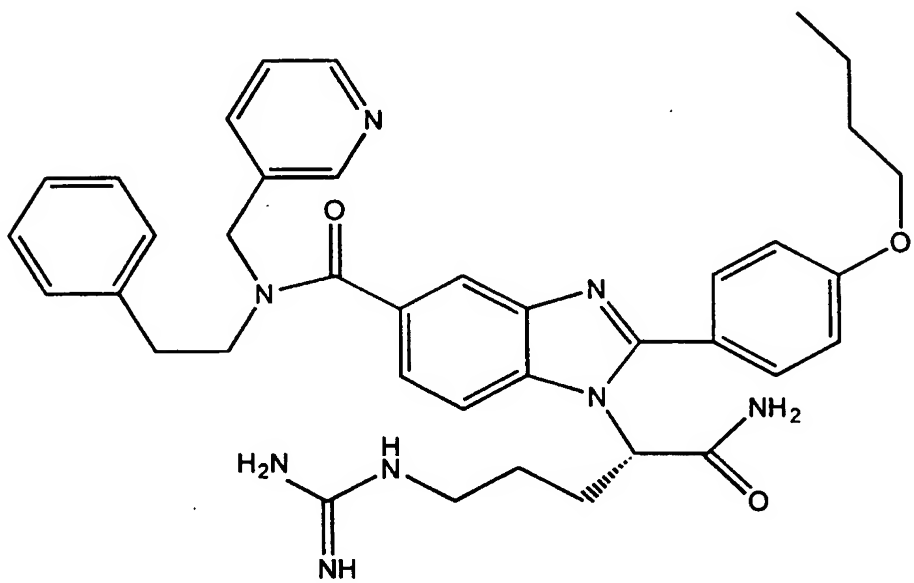
In Compound K, R_1 is derivatized with arginine, R_2 is derivatized with 4-(4-chlorophenyl)-4-hydroxypiperidine and R_3 is derivatized with 4-*t*-butylbenzaldehyde:



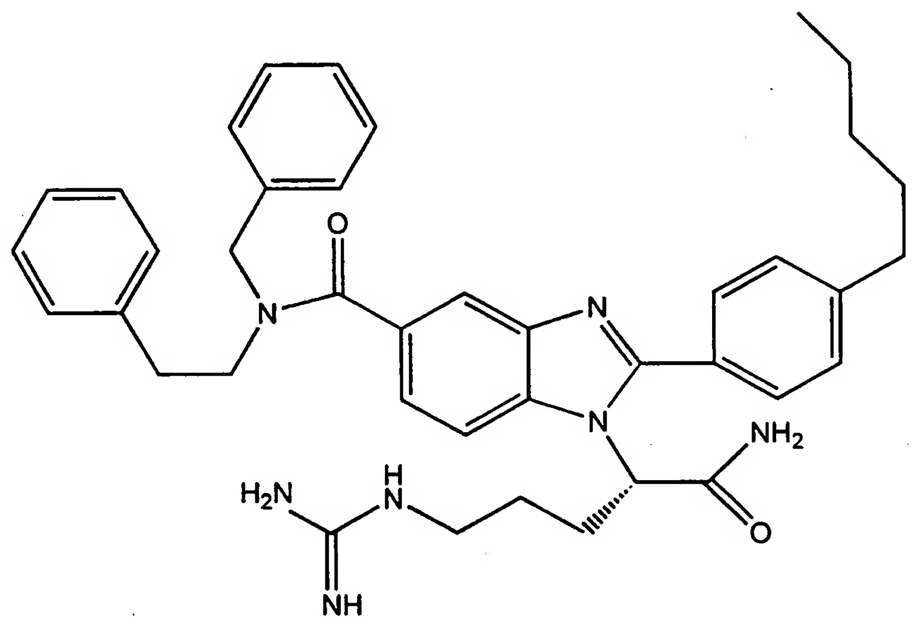
In Compound L, R_1 is derivatized with arginine, R_2 is derivatized with N-(3-pyridylmethyl)-N-phenethylamine and R_3 is derivatized with 4-*t*-butylbenzaldehyde:



In Compound M, R_1 is derivatized with arginine, R_2 is derivatized with N-(3-pyridylmethyl)-N-phenethylamine and R_3 is derivatized with 4-butoxybenzaldehyde:



In Compound N, R_1 is derivatized with arginine, R_2 is derivatized with N-benzylphenylethylamine and R_3 is derivatized with 4-amylbenzaldehyde:



The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

Synthesis of compounds

A. Synthesis of HP-228

HP-228 was synthesized essentially as described in U.S. Patent No. 5,420,109.

Briefly, 100 mg MBHA resin containing a *t*-Boc Gly derivative was added to a reaction vessel suitable for solid phase peptide synthesis (Houghten, Proc. Natl. Acad. Sci. USA 82:5131 (1985)). The following conditions were used for peptide synthesis: coupling was performed in 6-fold excess in N,N-dimethylformamide (DMF) with 0.2 M N-hydroxybenzotriazole(HOBt) and 0.2 M N,N-diisopropylcarbodiimide (DIC) for 90 minutes; activation was performed with 5% diisopropylethylamine (DIEA) in methylene chloride (DCM) for three washes of 2 min; deprotection was performed with 55% trifluoroacetic acid (TFA) for 30 min; washes were performed with DCM and isopropanol; the ninhydrin test was run after washing with DMF, DCM and methanol; acetylation was performed with acetylimidazole in 40-fold excess DCM for 4 hr; and cleavage was performed with hydrofluoric acid (HF) and anisole for 90 min.

Peptide synthesis was carried out with the sequential steps of activation, coupling of amino acid, ninhydrin test, deprotection and washing, and the steps were repeated for addition of a new amino acid at each cycle. The amino acids were coupled in the order D-Trp, L-Arg, D-Phe, L-His, L-Gln and L-Nle. The peptide was acetylated and the DNP protecting group was removed from His using 2.5% thiophenol in DMF followed by removal of formyl protecting groups in 25% HF in dimethylsulfide. The peptide was cleaved from the resin and processed as described previously (United States Patent No. 5,420,109). The resulting peptide was approximately 80 to 90% pure.

B. Preparation of HP-467

Compound HP-467 was prepared by iodinating HP-228, described above. The iodinated compound can be radiolabeled or unlabeled.

For iodination, 100 µl 2 N H_2SO_4 and 400 µl 0.5 M $CuSO_4$ was added to 12 mg Zn powder, and the components were allowed to react with periodic mixing for 30 to 45 min, with venting, until bubbling stopped. The grains were washed twice with water. For unlabeled peptide, 7.12 µl of 0.67 mM NaI (0.0047 µmol) was added to the reaction vial. For radiolabeled peptide, 0.0047 µmol of $Na^{125}I$ was added to the reaction vial. Approximately 1/8 of the copper grains were added to the vial, and the vial was vortexed for 1 minute. The reaction was carried out vented at room temperature for 3 hr with periodic mixing.

Samples were analyzed on a Vydac 218TP54 C-18 column and were monitored at 214 nm. Buffer Aawas 0.05% TFA in water, and Buffer B was 0.05% TFA in acetonitrile. Samples were resolved using a 2% per minute gradient from 5 to 55% Buffer B in 25 min.

Using this method, ^{125}I-HP 467 was routinely labeled to a specific activity of 2000 Ci/mmol. These results demonstrate that HP-467 can be iodinated to generate unlabeled iodo-peptide or high specific activity radiolabeled iodo-peptide.

EXAMPLE II

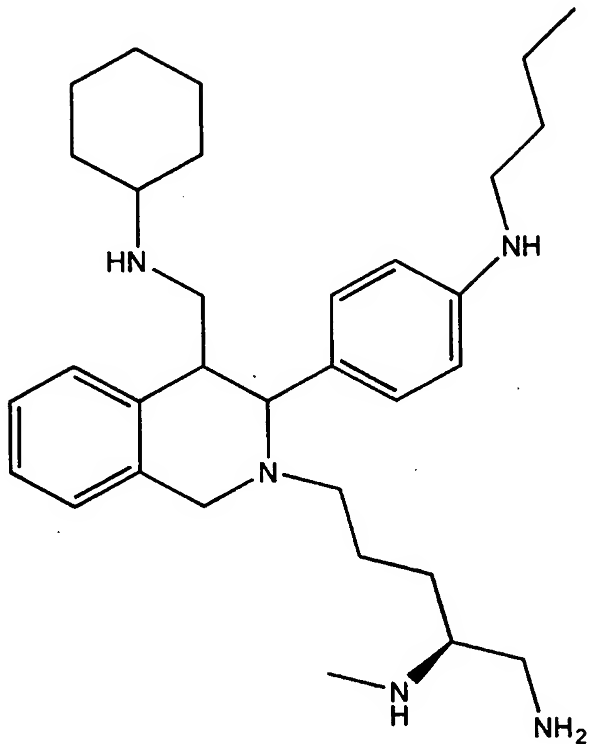
Induction of erections upon administration of HP-228

A. Induction of erections in rats

Adult male rats were housed 2 to 3 per cage and were acclimated to the standard vivarium light cycle (12 hr. light, 12 hr. dark), rat chow and water for a least a week prior to testing. All experiments were performed between 9 a.m. and noon and rats were placed in cylindrical, clear plexiglass chambers during the 60 minute observation period. Mirrors were positioned below the chambers to improve viewing.

Observations began 5 minutes after an intraperitoneal injection of either saline or compound. Two observers counted the number of grooming motions, stretches, yawns and spontaneously occurring penile erections not elicited by genital grooming, and then recorded them as they occurred for a total of 60 minutes. Each observer recorded behaviors of 6 rats at the same time. The observers were unaware of the treatment and animals were tested once, with n = 6 in each group. HP-228 was used as a positive control for penile erections.

Upon observation, an average of 4.8 erections were observed per rat per hour when HP-228 was injected alone, compared to 0.2 for vehicle (PBS), thus demonstrating that HP-228 can induce erections in rats. By comparison, IP injection with agonist TRG 2411 #203 alone (shown below) increased the average number of erections observed to 2.7.



B. Dose-response curve in rats

This example describes a dose-response correlation for using HP-228 to induce erections in rats.

Adult male Sprague-Dawley rats weighing 220 to 250 g or adult male C57/BL6 mice (Harlan Laboratories; Indianapolis IN) were injected intraperitoneally (IP) with 1 ml/kg body weight HP-228 solution or PBS as a negative control. Concentrations of HP-228 were 0.45, 0.9, 1.8, 3.6 and 7.2 mg/ml/kg (n = 4 to 6 animals). The animals were allowed 10 minutes to acclimate to the surroundings before observation. The animals were then observed for 60 minutes for penile erections not induced by grooming. Yawning, stretching, grooming behaviors were also recorded.

mg/ml/kg HP-228	avg. erections observed / hour
0.0 (control)	0.25
0.45	2.80
0.9	2.60
1.8	3.67
3.6	7.00
7.2	5.75

These results demonstrate a dose-response effect by HP-228 for inducing erections in rats.

C. Dose-response curve in mice

This example describes a dose-response correlation for using HP-228 to induce erections in mice (n = 3 to 5). Age-matched C57/BL6 mice (Harlan Laboratories; Indianapolis IN) weighing 20 to 25 g were injected (100 μl/mouse) intraperitoneally essentially as described above and observed.

μg/mouse HP-228	avg. erections observed / hour
0 (control)	0.33
25	3.00
50	6.67
100	5.33
250	3.00

These results demonstrate that HP-228 is also erectogenic in mice.

EXAMPLE III

Melanocortin Receptor Assay

This example describes methods for assaying ligand binding to MC receptors.

All cell culture media and reagents were obtained from GibcoBRL (Gaithersburg MD), except for COSMIC CALF SERUM (HyClone; Logan UT). HEK 293 cell lines were transfected with human MC receptors hMCR-1, hMCR-3 and hMCR-4 (Gantz et al., Biochem. Biophys. Res. Comm. 200:1214-1220 (1994); Gantz et al., J. Biol. Chem. 268:8246-8250 (1993); Gantz et al. J. Biol. Chem. 268:15174-15179 (1993); Haskell-Leuvano et al., Biochem. Biophys. Res. Comm. 204:1137-1142 (1994)). Vectors for constructing an hMCR-5-expressing cell line were obtained and a line of HEK 293 cells expressing hMCR-5 was constructed (Gantz, *supra*, 1994). Receptor hMCR-5 has been described previously (Franberg et al., Biochem. Biophys. Res. Commun. 236:489-492 (1997); Chowdhary et al., Cytogenet. Cell Genet. 68:1-2 (1995); Chowdhary et al., Cytogenet. Cell Genet. 68:79-81 (1995)). HEK 293 cells were maintained in DMEM, 25 mM HEPES, 2 mM glutamine, non-essential amino acids, vitamins, sodium pyruvate, 10% COSMIC CALF SERUM, 100 units/ml penicillin, 100 μg/ml streptomycin and 0.2 mg/ml G418 to maintain selection.

Before assaying, cells were washed once with phosphate buffered saline (PBS without Ca^{2+} and Mg^{2+}) and stripped from the flasks using 0.25% trypsin and 0.5 mM EDTA. Cells were suspended in PBS, 10% COSMIC CALF SERUM and 1 mM $CaCl_2$. Cell suspensions were prepared at a density of 2×10^4 cells/ml for HEK 293 cells expressing hMCR-3, hMCR-4 or hMCR-5, and 1×10^5 cells/ml for HEK 293

cells expressing hMCR-1. Suspensions were placed in a water bath and allowed to warm to 37°C for 1 hr.

Binding assays were performed in a total volume of 250 µl for HEK 293 cells. Control and test compounds were dissolved in PBS pH 7.4. Custom labeled ligand ^{125}I-HP 467 (50,000 dpm, 2000 Ci/mmol) (Amersham; Arlington Heights IL) was prepared in 50 mM Tris, pH 7.4, 2 mg/ml BSA, 10 mM $CaCl_2$, 5 mM $MgCl_2$, 2 mM EDTA and added to each tube. To each tube was added 4 x 10^3 HEK 293 cells expressing hMCR-3, hMCR-4 or hMCR-5, or 2 x 10^4 cells expressing hMCR-1. Assays were incubated for 2.5 hours at 37°C.

GF/B filter plates (Packard Instr.; Meriden CT) were prepared by soaking for at least one hour in 5 mg/ml BSA and 10 mM $CaCl_2$. Assays were filtered using a Brandel 96-well cell harvester (Brandel Inc.; Gaithersburg MD). The filters were washed four times with cold 50 mM Tris, pH 7.4; the filter plates were dehydrated overnight; and 35 µl of MICROSCINT (Packard Instr.; Meriden CT) was added to each well. Filter plates were counted using a Packard Topcount (Packard Instr.; Meriden CT) and data analyzed using Microsoft EXCEL v5.0a (Microsoft Corp.; Redmond WA) and XLfit 2.0 (ID Business Solutions; Guildford, Surrey UK)

To assay compounds, binding assays were performed in a 96-well format. HP 467 was prepared in 50 mM Tris, pH 7.4, and ^{125}I-HP 467 was diluted to give 100,000 dpm per 50 µl. A compound was added to the well in 25 µl aliquots. A 25 µl aliquot of ^{125}I-HP 467 was added to each well. A 0.2 ml aliquot of suspended cells was added to each well to give the cell numbers indicated above, and the cells were incubated at 37°C for 2.5

hours. Cells were harvested on GF/B filter plates as described above and counted.

EXAMPLE IV

cAMP Assay for Melanocortin Receptors

This example describes methods for assaying cAMP production from G-protein-coupled MC receptors.

HEK 293 cells individually expressing MCR-1, MCR-3, MCR-4 or MCR-5 were used. Cells were plated at 20,000 cells per well in a 96-well plate coated with collagen (Beckton Dickinson; Bridgeport NJ). The next day, cells were pretreated with 88 µl of 0.4 mM 3-isobutyl-1-methylxanthine (IBMX) in low serum medium containing DMEM, 25 mM HEPES and 0.1% COSMIC CALF SERUM. IBMX is an inhibitor of cAMP phosphodiesterase. The pretreatment was carried out for 10 minutes at 37°C.

Following pretreatment, 12.5 µl of diluted compound was added to the wells and cells were incubated for 15 min at 37°C. Cells were lysed by adding 25 µl saponin lysis buffer (130 mM KCl, 1 mM KH_2PO_4, 2.5 mM $MgSO_4$, 15 mM sucrose, 20 mM HEPES, 2% BSA, 0.8% saponin) and incubating 2 to 5 minutes. Plates were covered and stored at -20°C.

The cAMP concentration was determined by ELISA. Briefly, 96 well ELISA plates were coated with goat anti-cAMP antibody in PBS for 12 to 72 hr at 4°C. 50 µl of sample was mixed with 50 µl of cAMP ELISA buffer containing 1% bovine serum albumin, 10% heat inactivated donor horse serum, 1% normal mouse serum and 0.05% TWEEN-20 in PBS, and the diluted sample was added to the coated ELISA plate. Standards of known concentrations of cAMP

were added to separate wells. Then, cAMP-conjugated horseradish peroxidase (cAMP-HRP) was added to each well at 25 µl of 16 ng/ml. The plates were incubated for 3 hr at room temperature. Plates were washed and the binding of cAMP-HRP was detected with 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide using standard immunoassay procedures.

EXAMPLE V

Melanocortin Receptor Binding and Activation Profile of Compounds

Various compounds were tested for *in vitro* activity and binding to melanocortin receptors. MC-3 stimulatory effect is also described in terms of percentage of stimulation obtained by adding HP-228 at 0.8 μM. The EC_{50} values are the concentration of ligand necessary to achieve 50% of the maximum increase by that ligand in an assay. The IC_{50} values are the concentration giving 50% inhibition of binding of ^{125}I-HP 467.

		EC_{50} (μM) values		
Compound	**% of HP-228**	**MC-3**	**MC-1**	**MC-4**
A	89%	7.65	0.47	
B	115%	1.33		12
C	92%	1.4		10.6
D	140%	1.7		2.8
E	59%	6.7	0.43	19.9

Compounds K to N were also tested for stimulatory effect on MC-3, MC-1, MC-4 and MC-5.

		EC_{50} (μM) values		
Compound	**% of HP-228**	**MC-3**	**MC-1**	**MC-4**
K	91%	1.75	0.38	0.08
L	350%	4.69	0.22	6.35
M	89%	3.67		
N	229%	2.32		5.90

These results show that the Compounds A to E and K to N can act as MC-3 agonists, often exceeding the stimulatory activity of HP-228 in comparable assays. Moreover, Compounds B to E, L and N each have a greater stimulatory effect on MC-3 than at least one other MC receptor, thus demonstrating MC-3-preferring activity.

Compounds F to J (structures not shown) were tested for competitive binding to MC-3, MC-1, MC-4 and MC-5.

		IC_{50} (μM) values		
Compound	**MC-3**	**MC-1**	**MC-4**	**MC-5**
F	2.664	1.668	2.849	0.343
G	4.015	4.475	5.808	0.325
H	1.455	0.645	4.682	0.269
I	0.405	3.385	2.164	0.172
J	10.863	15.496	20.160	28.049

These results demonstrate that compounds such as Compound J can have stronger binding to MC-3 compared to other melanocortin receptors. In addition, although Compound I does bind MC-5, it binds MC-3 more tightly than MC-1 or MC-4 and is therefore considered MC-3-preferring.

EXAMPLE VI

Association of MC-3 with erectile response

This example shows that erectogenesis is associated with MC-3.

Sprague-Dawley rats (n = 5) received 1.8 mg/kg HP-228 or PBS via IP injection. The rats also received via ICV injection a dose of PBS or MC-3-specific antagonist at 10 μg/5 μl. The compound designated "TRG 6600 #3_4" is a specific MC-3 antagonist but is active to the MC-4 receptor. After administration, the rats were observed as described above. All rats received an ICV injection followed by an IP injection 5 to 10 minutes later.

In the negative control rats, receiving only PBS, 0.3 erections per hour were observed. In the positive control rats, receiving HP-228 but no MC-3 antagonist, 3.8 erections per hour were observed. In rats receiving HP-228 and the MC-3-specific antagonist, the number of erections observed decreased to 0.8 per hour, demonstrating that the HP-228-induced erections were antagonized by the MC-3-specific antagonist, thereby identifying MC-3 as the MC receptor associated with erectogenesis. No erections were observed in the rats receiving the MC-3-specific antagonist and no HP-228. The MC-3-specific antagonist is also known to have MC-4 agonist properties, which were reflected by significantly reduced food consumption.

EXAMPLE VII

Effects of oxytocin receptor and dopamine receptor antagonists on HP-228 erectile effects

These examples show the effects of antagonists to oxytocin and dopamine receptors on the erectogenic activity of HP-228.

In both examples, male Sprague-Dawley rats, about 200 to 225 g, were acclimated for 5 days in a reverse light:dark cycle room (dark 10 a.m. to 10 p.m.) before being aseptically ICV cannulated. Rats with indwelling ICV cannulae were housed one per cage. After surgery, 4 days were allowed for recovery and 10 days total were allowed for adaptation to the light cycle. Rats were caged individually after surgery and body weights at the time of treatment averaged 250 g. Otherwise, the procedure is essentially the same as in Example II.

a. Oxytocin pathway

Oxytocin is a potent erectogenic compound when administered ICV. The erectile effects of oxytocin can be blocked by specific receptor antagonists such as vasotocin, $d(CH_2)_5$-Tyr(Me^2)-Orn^8-Vasotocin.

Treatment was between 10 a.m. and 1 p.m. The rats had an ICV pre-treatment injection of vasotocin, vehicle control (PBS). Vasotocin (Peninsula Laboratories, San Carlos CA) was administered ICV at 100 ng/rat. Oxytocin (Sigma, St. Louis MO) was administered ICV at 30 ng/rat (10 μl/rat PBS). Ten minutes later, IP injections of HP-228 or vehicle were given. HP-228 was

administered IP at 2 mg/kg in 3 ml/kg PBS. One group of animals received ICV injections of vasotocin and oxytocin ICV injections of 5 μl/rat each at double concentrations for a total of 10 μl injected ICV. In this group, the rats received vasotocin first, followed by oxytocin.

The animals were observed for grooming, stretching, yawning and penile erections for 60 minutes, starting 5 minutes after treatment. Erections per hour and responder rate of animals (e.g., 7 rats responding out of 8 rats) are shown:

treatment		**erections per hour (rate)**
Oxytocin (icv),	PBS (ip)	3.9 (7/8)
PBS (icv),	HP-228 (ip)	4.9 (7/8)
PBS (icv),	PBS (ip)	0.1 (1/8)
Vasotocin (icv),	PBS (ip)	0.2 (2/8)
Vasotocin (icv) + Oxytocin (icv)		1.0 (4/8)
Vasotocin (icv),	HP-228 (ip)	5.6 (8/8)

Oxytocin and HP-228 showed significant erectile response (response rate 88%-100% of animals) compared to vehicle or vasotocin. Vasotocin alone had no significant erectile effect, but antagonized the erectile effects of oxytocin (1.0 compared to 3.9). Significantly, vasotocin did not reduce the erectile activity of HP-228 (5.6 compared to 4.9).

These results indicate that the oxytocin receptor antagonist vasotocin significantly antagonizes the erectile effect of oxytocin, but does not affect the erectile effect of HP-228, indicating that the mechanism of HP-228 does not involve central oxytocin.

b. Dopamine pathway

Apomorphine is a potent erectogenic compound upon peripheral or central administration. The erectile effects of apomorphine can be blocked by specific dopamine receptor (D2, D3) antagonists such as sulpiride.

Treatment was between 9:30 a.m. and noon. Each animal received an initial IP injection of vehicle (3 ml/kg PBS) or sulpiride. Sulpiride (Sigma) was administered IP at 50 mg/kg (6 ml/kg IP). Ten minutes later, the animals received a final IP injection of HP-228 or vehicle or a SC injection of apomorphine. HP-228 was administered IP at 2 mg/kg in 3 ml/kg PBS. Apomorphine (Sigma) was administered SC at 0.1 mg/kg (1 ml/kg sterile water). The animals were observed as in Example VII.a. Erections per hour and rate of animals responding are shown:

treatment		**erections per hour (rate)**
PBS (ip),	apomorphine (sc)	4.0 (6/6)
PBS (ip),	HP-228 (ip)	7.3 (5/5)
PBS (ip),	PBS (ip)	0.5 (2/4)
sulpiride (ip),	PBS (ip)	0.0 (0/2)
sulpiride (ip),	apomorphine (sc)	1.5 (3/4)
sulpiride (ip),	HP-228 (ip)	6.7 (4/4)

Apomorphine and HP-228 showed significant erectile response (response rate of 100% of animals) compared to vehicle or sulpiride. Sulpiride alone had no significant erectile effect, but antagonized the erectile effects of apomorphine (1.5 compared to 4.0). Significantly, sulpiride did not significantly reduce the erectile activity of HP-228 (6.7 compared to 7.3).

These results indicate that the dopamine receptor (D2, D3) antagonist sulpiride significantly antagonizes the erectile effect of apomorphine, but does not significantly affect the erectile activity of HP-228, indicating that the mechanism of HP-228 does not involve dopaminergic activation.

EXAMPLE VIII

Further association of MC-3 with HP-228-mediated erectile response

This example further shows that erectogenesis is associated with MC-3. A compound designated "TRG 6601 #15_2" is a specific MC-3 antagonist but lacks *in vitro* or *in vivo* activity at the MC-4 receptor.

Rats were obtained and cannulated essentially as described in Example VII. Rats received a 10 μl injection ICV containing antagonist "TRG 6601 #15_2" or vehicle alone. The compound was administered as a 1 mg/ml solution in 5% EtOH/PBS at a dose of 10 or 50 μg/rat. Five minutes later, the rats were injected IP with either PBS (1 ml/kg) or HP-228 (2 mg/kg/ml). The animals were observed as in Example VII. Erections per hour and rate of animals responding are shown:

treatment		**erections per hour**	**(rate)**
PBS (icv),	HP-228 (ip)	4.7	(6/6)
PBS (icv),	PBS (ip)	0.2	(1/5)
50 μg antagonist (icv),	PBS (ip)	0.2	(1/5)
10 μg antagonist (icv),	PBS (ip)	0.2	(1/5)
50 μg antagonist (icv),	HP-228 (ip)	0.5	(1/2)
10 μg antagonist (icv),	HP-228 (ip)	5.8	(6/6)

Rats receiving HP-228 had a much higher erectile rate than rats receiving PBS alone or the antagonist at 10 μg or 50 μg. Significantly, rats receiving HP-228 and 50 μg antagonist had a much lower erectile rate than rats receiving HP-228 alone (0.5 compared to 4.7). However, the antagonist did not reduce the erectile rate when administered at only 10 μg (5.8 compared to 4.7), showing that the compound's antagonism is dose-dependent. These results further show that MC-3 is responsible for mediating melanocortin-mediated penile erections. In addition, the lack of MC-4 activity is shown by lack of MC-4-related feeding effect after ICV injection, even at a relatively high dose of 50 μg/rat.

EXAMPLE IX

Preparation of 2-morphilino-7-alkyl-11-alkylaminocarbonyl -5H-benzimidazol[1,2,d][1,4]benzodiazepin-6(7H)-one [1-(1-aminocarbonyl-2-phenyl)ethyl-2-substituted-benzimid azol-5-yl]carboxamides

1. Coupling of N-protected amino acid to MBHA resin

1.0 g of MBHA resin (1.3 meq/g) was placed in a porous polypropylene packet (Tea-bag, 60mm x 60mm, 65μ). The packet was washed with 5% DIEA/DCM (2 X 60 mL) in a 125 mL plastic bottle. DMF (80 mL), Boc-phenylalanine (4.24g, 16 mmol), DIC (3.03g, 24 mmol), HOBt (2.16g, 16 mmol) were added sequentially. After shaking for 24 hours, the packet was washed alternately with DMF (80 mL) and MeOH (80 mL) for 3 cycles followed by DCM (80 mL) and MeOH (80 mL). The packet was dried in air for 2 hours. The packet was shaken with 55% TFA/DCM (80 mL) at room temperature for 40 minutes and washed

with DCM (3 X 80 mL), 5% DIEA/DCM (2 X 80 mL) and MeOH (80 mL).

2. N-Arylation with 4-fluoro-3-nitrobenzoic acid.

The packet was heated in a solution of 4-fluoro-3-nitrobenzoic acid (2.96g, 16 mmol) and DIEA (2.02g, 16 mmol) in N-methylpyrrolidinone (80 mL) at 70° C for 24 hours. The packet was washed alternately with DMF (80 mL) and MeOH (80 mL) for 3 cycles followed by washing with DCM (80 mL) and MeOH (80 mL). The packet was dried in air overnight.

3. Coupling amine onto resin-bound carboxylic acid.

The packet was shaken with a solution of morpholine (1.40 g, 16 mmol), DIC (3.03g, 24 mmol) and HOBt (2.16g, 16 mmol) in DMF (80 mL) for 24 hours. The packet was washed alternately with DMF (80 mL) and MeOH (80 mL) for 3 cycles followed by DCM (80 mL) and MeOH (80 mL). The packet was dried in air overnight.

4. Reduction of the nitro group to amine.

The packet was shaken with a 2.0 M solution of tin(II) chloride dihydrate in N-Methylpyrrolidinone (80 mL) for 24 hours at room temperature. The packet was washed with DMF (4 X 80 mL), 10% DIEA/DCM (4 X 80 mL), MeOH, (2 X 80 mL), DMF (80 mL), MeOH (80 mL), DCM (2 X 80 mL) and MeOH (2 X 80 mL) and dried in air overnight.

5. Reaction with aldehydes to form benzimidazoles.

The packet was cut open and the resin was suspended in N-methylpyrrolidinone (30 mL). The suspension was distributed equally into 68 wells of a microtiter plate (2mL X 96). N-Methylpyrrolidinone (240 μL), acetic acid (185 μL) and a solution of corresponding aldehyde (see list below) in N-methylpyrrolidinone (100 μL X 1.0 M) were added to each well. The plate was tightly capped, shaken and incubated at 67° C for 48 hours. The resin was washed alternately with DMF (3 X 1 mL/well) and MeOH (2 X 1 mL/well), DCM/t-BuOMe (50%, 2 X 1 mL/well) and MeOH (2 X 1 mL/well). The plate was dried in air overnight and under vacuum for 4 hours. The plate was treated with gaseous HF at room temperature for 2 hours. After complete removal of HF under a nitrogen stream followed and by vacuum, the plate was extracted with AcOH (4 x 0.5 mL/well). The extraction solutions were lyophilized.

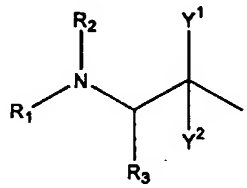
Although the invention has been illustrated by the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

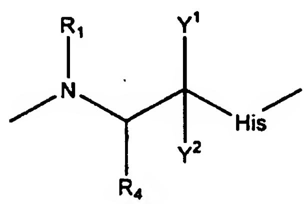
We claim:

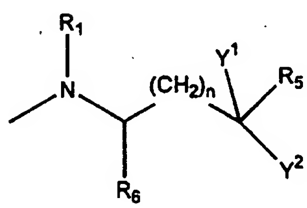
1. A method for treating sexual dysfunction in a subject, comprising the step of administering to the subject an effective dose of the compound

$$X_1\text{-}X_2\text{-(D)Phe-Arg-(D)Trp-}X_3$$

wherein

X_1 is , $COCH_3$, H or absent;

X_2 is , His, $COCH_3$ or H; and

X_3 is  or R_5; wherein

R_1 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, COO-t-butyl, $COOCH_2Ph$, CH_2CO-(polyethylene glycol) or A;

R_2 is H, $COCH_3$, C_2H_5 or CH_2Ph;

R_3 is a linear alkyl group having 1 to 6 carbon atoms or a branched or cyclic alkyl group having 3 to 6 carbon atoms;

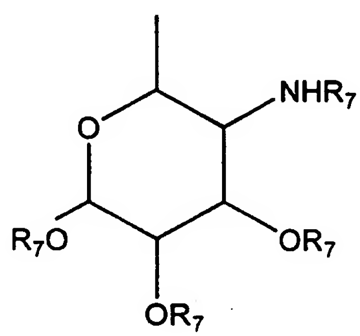
R_4 is $(CH_2)_m$-$CONH_2$, $(CH_2)_m$-$CONHR_1$ or $(CH_2)_m$-CONHA;

R_5 is OH, OR_3, NH_2, SH, $NHCH_3$, $NHCH_2Ph$ or A;

R_6 is H or R_3;

R_7 is H, $COCH_3$, C_2H_5, CH_2Ph, COPh, COO-*t*-butyl, $COOCH_2Ph$ or CH_2CO-(polyethylene glycol);

Ph is C_6H_5; m is 1, 2 or 3; n is 0, 1, 2 or 3; Y^1 and Y^2 are independently hydrogen atoms, or are taken together to form a carbonyl or thiocarbonyl; and A is



2. The method of claim 1, wherein X_1 is selected from the group consisting of norleucine and Ac-norleucine.

3. The method of claim 1, wherein X_1 is selected from the group consisting of norvaline, Ac-norvaline, leucine, Ac-leucine, isoleucine and Ac-isoleucine.

4. The method of claim 1, wherein X_2 is selected from the group consisting of Gln-His and His.

5. The method of claim 1, wherein X_3 is selected from the group consisting of Gly and Gly-NH_2.

6. The method of claim 1, wherein R_1 is selected from the group consisting of H, C_2H_5 and CH_2Ph.

7. The method of claim 1, wherein R_1 and R_2 are independently selected from the group consisting of $COCH_3$ and H.

8. The method of claim 1, wherein R_5 is NH_2.

9. The method of claim 1, wherein R_5 is covalently bound to X_1, forming a cyclic peptide.

10. The method of claim 1, wherein the compound HP-228, having the structure Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2.

11. The method of claim 1, wherein the (D)Phe is iodinated in the *para* position.

12. The method of claim 11, wherein the compound is Ac-Nle-Gln-His-(*para*-iodo-(D)Phe)-Arg-(D)Trp-Gly-NH_2.

13. The method of claim 1, wherein the compound is selected from group consisting of

(D)Phe-Arg-(D)Trp,
Ac-(D)Phe-Arg-(D)Trp,
(D)Phe-Arg-(D)Trp-NH_2 and
Ac-(D)Phe-Arg-(D)Trp-NH_2.

14. The method of claim 1, wherein the compound is selected from group consisting of

(cyclohexyl)Gly-Gln-His-(D)Phe-Arg-(D)Trp-Gly,
Ac-(cyclohexyl)Gly-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2 and
cyclo(His-(D)Phe-Arg-(D)Trp).

15. The method of claim 1, wherein the compound is selected from group consisting of

Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2,
His-(D)Phe-Arg-(D)Trp-Gly,
His-(D)Phe-Arg-(D)Trp-Gly-NH_2,
Ac-His-(D)Phe-Arg-(D)Trp-NH_2,
His-(D)Phe-Arg-(D)Trp-OH,
His-(D)Phe-Arg-(D)Trp,
His-(D)Phe-Arg-(D)Trp-NH_2,
Ac-His-(D)Phe-Arg-(D)Trp-OH and
Ac-His-(D)Phe-Arg-(D)Trp-Gly-NH_2.

16. The method of claim 1, wherein the compound is selected from group consisting of

Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-OH,
Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-OC_2H_5,
Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH-NH_2,
Ac-Nle-Asn-His-(D)Phe-Arg-(D)Trp-Gly-NH_2,
Ac-Nle-Asn-His-(D)Phe-Arg-(D)Trp-Gly-OH,
Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-$NHCH_2CH_2Ph$,
Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly-$NHCH_2Ph$,
Nle-Gln-His-(D)Phe-Arg-(D)Trp-Gly,
N——————————————————O
Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2,
Ac-Gln-His-(D)Phe-Arg-(D)Trp-Gly-NH_2,
Ac-Nle-Gln-His-(D)Phe-Arg-(D)Trp-NH_2,
Ac-His-(D)Phe-Arg-(D)Trp(CH_2)-(NAc)Gly-NH_2 and
His-(D)Phe-Arg-(D)Trp(CH_2)-(NAc)Gly.

17. The method of claim 1, wherein the subject is male.

18. The method of claim 17, wherein the dysfunction is erectile dysfunction.

19. The method of claim 1, wherein the subject is female.

20. The method of claim 19, wherein the dysfunction is sexual arousal disorder.

21. A method for selecting a melanocortin receptor 3 (MC-3) ligand, comprising the steps of

(a) contacting a compound with an MC-3 receptor; and

(b) determining whether the compound modulates the activity of the receptor;

whereby the compound is selected if the compound modulates the activity of the receptor.

22. The method of claim 21, wherein the modulation of the receptor's activity is determined by a cAMP assay.

23. The method of claim 21, wherein the ligand is an MC-3 agonist that stimulates the activity of the receptor.

24. The method of claim 23, wherein the ligand has an EC_{50} less than 1 μM.

25. The method of claim 24, wherein the ligand has an EC_{50} less than 100 nM.

26. The method of claim 22, wherein HP-228 is used as a positive control.

27. The method of claim 26, wherein the ligand has at least 50% of the stimulatory effect of HP-228.

28. The method of claim 21, wherein the ligand is an MC-3 antagonist that decreases the activity of the receptor.

29. The method of claim 28, wherein the ligand decreases the activity of the receptor in the presence of HP-228.

30. The method of claim 21, further comprising the step of

(c) determining whether the ligand is MC-3-preferring compared to a second melanocortin receptor;

whereby the compound is selected if the compound is an MC-3 ligand and MC-3-preferring.

31. The method of claim 30, wherein the ligand is MC-3-preferring if the ligand has a lower EC_{50} for MC-3 than for the second MC.

32. The method of claim 31, wherein the ligand's EC_{50} for MC-3 is less than 20% of the ligand's EC_{50} for the second MC.

33. The method of claim 2, wherein the ligand is MC-3-preferring if the ligand has a lower IC_{50} for MC-3 than for the second MC.

34. The method of claim 33, wherein the ligand's IC_{50} for MC-3 is less than 20% of the ligand's IC_{50} for the second MC.

35. The method of claim 30, wherein the second melanocortin receptor is melanocortin receptor 1 (MC-1).

36. The method of claim 30, wherein the second melanocortin receptor is melanocortin receptor 2 (MC-2).

37. The method of claim 30, wherein the second melanocortin receptor is melanocortin receptor 4 (MC-4).

38. The method of claim 30, wherein the second melanocortin receptor is melanocortin receptor 5 (MC-5).

39. A method for screening a library of compounds for MC-3 ligands, comprising the steps of

(a) selecting a compound from the library and

(b) performing the method of claim 21;

thereby screening the library for MC-3-specific ligands.

40. The method of claim 39, wherein the library has at least 50 compounds.

41. The method of claim 39, wherein the library has at least 200 compounds.

42. The method of claim 39, wherein the library has at least 1000 compounds.

43. A method for treating an MC-3-associated condition in a subject, comprising the step of administering to the subject an effective dose of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound obtained by performing the method of claim 39.

44. The method of claim 43, wherein the MC-3-associated condition is inflammation.

45. The method of claim 43, wherein the MC-3-associated condition is sexual dysfunction.

46. The method of claim 45, wherein the subject is male.

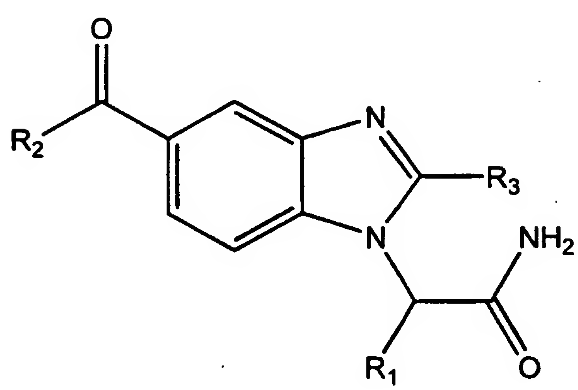
47. The method of claim 46, wherein the dysfunction is erectile dysfunction.

48. The method of claim 46, wherein the dysfunction is priapism.

49. The method of claim 45, wherein the subject is female.

50. The method of claim 49, wherein the dysfunction is sexual arousal disorder.

51. The method of claim 43, wherein ligand is a benzimidazole having the starting structure



, wherein

R_1 is derivatized with an amino acid;
R_2 is derivatized with a primary or secondary amine;
R_3 is derivatized with an aldehyde.

52. The method of claim 51, wherein R_1 is derivatized with arginine.

53. The method of claim 51, wherein R_2 is derivatized with a compound selected from the group consisting of phenethylbenzylamine and 1,2-diphenylethylamine.

54. The method of claim 51, wherein R_3 is derivatized with a compound selected from the group consisting of 4-*t*-butylbenzaldehyde, 4-*i*-propylbenzaldehyde and 4-butoxybenzaldehyde.

55. The method of claim 51, wherein the ligand is Compound A, wherein R_1 is derivatized with arginine, R_2 is derivatized with phenethylbenzylamine and R_3 is derivatized with 4-*t*-butylbenzaldehyde.

56. The method of claim 51, wherein the ligand is Compound B, wherein R_1 is derivatized with arginine, R_2 is derivatized with 1,2-diphenylethylamine and R_3 is derivatized with 4-butoxybenzaldehyde.

57. The method of claim 51, wherein the ligand is Compound C, wherein R_1 is derivatized with arginine, R_2 is derivatized with 1,2-diphenylethylamine and R_3 is derivatized with 4-*i*-propylbenzaldehyde.

58. The method of claim 51, wherein the ligand is Compound D, wherein R_1 is derivatized with arginine, R_2 is derivatized with phenethylbenzylamine and R_3 is derivatized with 4-*i*-propylbenzaldehyde.

59. The method of claim 51, wherein the ligand is Compound E, wherein R_1 is derivatized with arginine, R_2 is derivatized with 1,2-diphenylethylamine and R_3 is derivatized with 4-*t*-butylbenzaldehyde.

60. The method of claim 51, wherein the ligand is Compound K, wherein R_1 is derivatized with arginine, R_2 is derivatized with 4-(4-chlorophenyl)-4-hydroxypiperidine and R_3 is derivatized with 4-*t*-butylbenzaldehyde.

61. The method of claim 51, wherein the ligand is Compound L, wherein R_1 is derivatized with arginine, R_2 is derivatized with N-(3-pyridylmethyl)-N-phenethylamine and R_3 is derivatized with 4-*t*-butylbenzaldehyde.

62. The method of claim 51, wherein the ligand is Compound M, wherein R_1 is derivatized with arginine, R_2 is derivatized with N-(3-pyridylmethyl)-N-phenethylamine and R_3 is derivatized with 4-butoxybenzaldehyde.

63. The method of claim 51, wherein the ligand is Compound N, wherein R_1 is derivatized with arginine, R_2 is derivatized with N-benzylphenylethylamine and R_3 is derivatized with 4-amylbenzaldehyde.

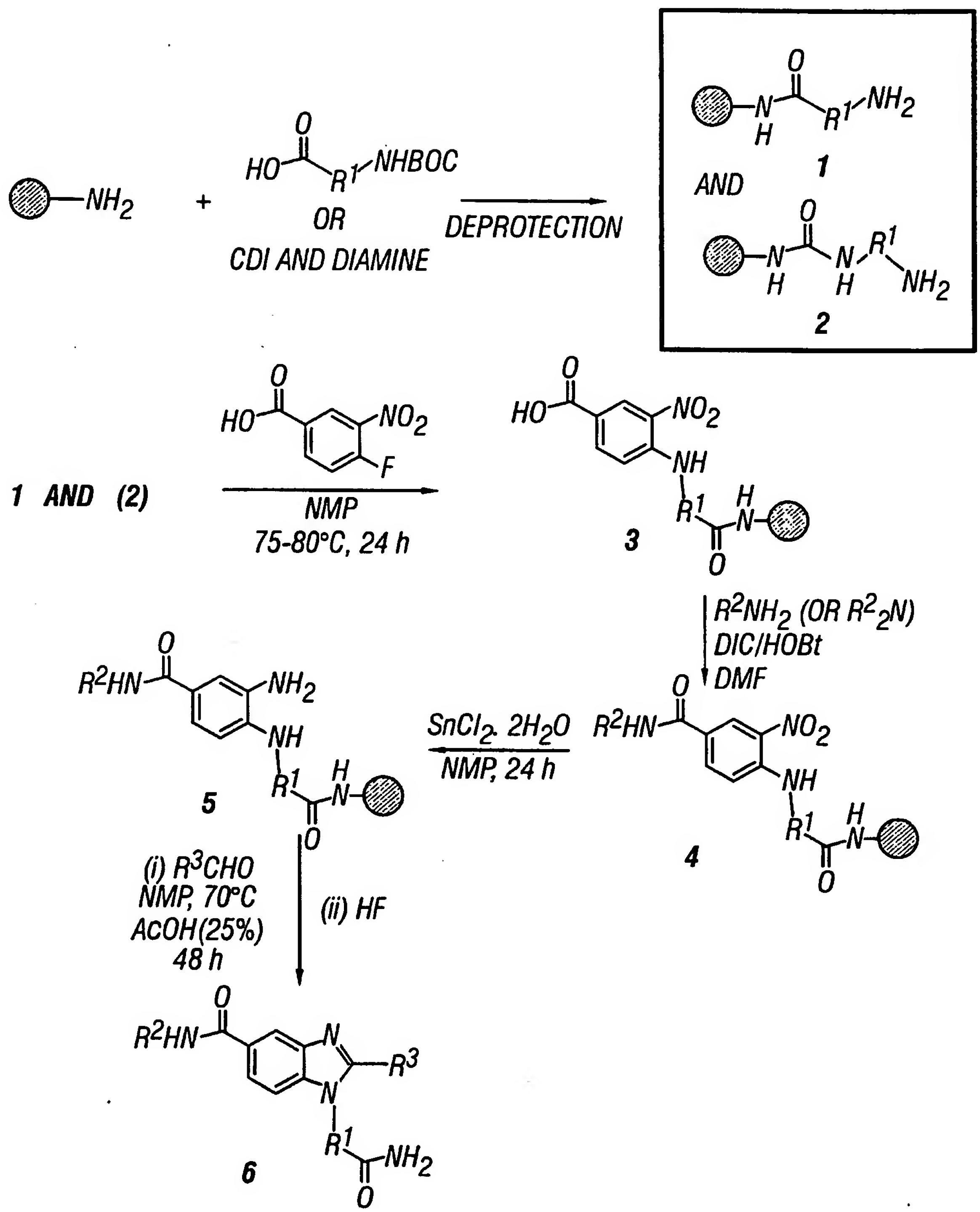

NH2
HO
O
R1
NHBOC
OR
CDI AND DIAMINE
DEPROTECTION
N
H
R1
NH2
1
AND
2
1 AND (2)
NO2
F
NMP
75-80°C, 24 h
NH
3
R2NH2 (OR R22N)
DIC/HOBt
DMF
R2HN
4
SnCl2. 2H2O
NMP, 24 h
5
(i) R3CHO
NMP, 70°C
AcOH(25%)
48 h
(ii) HF
R3
6

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19408

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 31/4184, 38/06, 38/07, 38/08; G01N 33/566
US CL :436/501; 514/17, 18, 394
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 436/501, 503; 514/17, 18, 394; 530/306, 312, 329, 330, 331

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,420,109 A (SUTO ET AL) 30 May 1995 (30/05/95).	1-63
Y	US 5,439,938 A (SNYDER ET AL) 08 August 1995 (08/08/95), column 2, lines 17-22, and claim 1.	1, 2, 4-8, 10, 17, 18
A	US 5,726,156 A (GIRTEN ET AL) 10 March 1998 (10/03/98).	1-63
X	US 5,837,521 A (CONE ET AL) 17 November 1998 (17/11/98), column 5, lines 7-29, and column 12, line 42 - column 13, line 35.	21-25, 28, 39-43
Y	US 5,889,056 A (HODSON ET AL) 30 March 1999 (30/03/99), column 2, lines 10-15, and column 6, lines 10-20.	1, 2, 4-8, 10, 17, 18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
29 AUGUST 2000

Date of mailing of the international search report
20 SEP 2000

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	US 6,054,556 A (HUBY ET AL) 25 April 2000 (25/04/00), column 4, line 38 - column 6, line 62.	21-25, 28, 30-35, 37-45
X,E	US 6,100,048 A (CONE ET AL) 08 August 2000 (08/08/00), column 3, lines 42-62, and claims 1-21.	21-25, 28, 30-43
Y	ABOU-MOHAMED et al. HP-228, A Novel Synthetic Peptide, Inhibits the Induction of Nitric Oxide Synthase *In Vivo* But Not *In Vitro*. The Journal of Pharmacology And Experimental Therapeutics. 1995, Volume 275, Number 2, pages 584-591, especially the Abstract, Figure 1, and page 590, column 1, second full paragraph.	1, 2, 4-8, 10, 17, 18
X	SCHIOTH et al. Characterization of the binding of MSH-B, HP-228, GHRP-6 and 153N-6 to the human melanocortin receptor subtypes. Neuropeptides. 1997, Volume 31, Number 6, pages 565-571, especially the Abstract and Table 2.	21-35, 37-42

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19408

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, DIALOG, CHEMICAL ABSTRACTS
search terms: hp 228, sexual dysfunction, erection, impotence, arousal, ejaculation, inflammation, priapism, melanocortin, mc3 receptor, nitric oxide synthase, structures of claims 1 and 51